(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number
WO 02/28869 A1

(51) International Patent Classification[7]: C07F 5/02, 9/38, C07C 229/22, A61K 31/14, 31/195, 33/22, A61P 35/00

(21) International Application Number: PCT/US01/31225

(22) International Filing Date: 4 October 2001 (04.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/238,320 5 October 2000 (05.10.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

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(54) Title: GAMMA-GLUTAMYL TRANSPEPTIDASE INHIBITORS

(57) Abstract: A method of inhibiting γ-glutamyl transpeptidase, (γGT) with an effective reversible inhibitor is disclosed. Exemplary compounds include L-2-amino-4-4-boronobutanoic acid (ABBA); 6,6-difluoro-5-oxo-t-phosphono-L-norleucine; and 6,6,6-trifluoro-5-oxo-L-norleucine. Included is a method for treating a condition or disease mediated by γ-GT, such as condition or disease characterized by increased expression of γ-GT. The method also includes treating a condition or disease where renal toxicity results from exposure to environmental, pharmacological, or other xenobiotic agents that form glutathione adducts or conjugates, which are then subsequently degraded in the kidney by γ-GT. In some instances, the condition or disease is a neoplasm.

GAMMA-GLUTAMYL TRANSPEPTIDASE INHIBITORS

FIELD

This invention relates to enzymatic inhibitors, particularly γ-glutamyl transpeptidase inhibitors.

BACKGROUND

Tumor cell resistance to anti-neoplastic agents is a major obstacle to the successful treatment of most cancers. Therefore, methods or techniques that compromise the ability of cancer cells to resist anti-neoplastic agents are potentially important to the treatment of cancer.

Glutathione is a ubiquitous tripeptide that has a prominent role in many cellular functions, including the detoxification of some biologically active molecules. Increased amounts of intracellular glutathione are associated with tumor resistance to chemotherapy or radiation therapy, and, correspondingly, tumors with low or repressed levels of intracellular glutathione are more sensitive to chemotherapy and radiation treatments. *See, e.g.*, Suzukake, K., et al., *Biochem. Pharmacol.*, 31:121-24 (1982); Green, J. A., et al., *Cancer Res.*, 44:5427-31 (1984).

The membrane-bound enzyme γ-glutamyl transpeptidase (γ-GT) plays a central role in the metabolism of glutathione. γ-GT is expressed in some cancers, may be induced in response to anticancer drugs or radiation therapy, and has been used as a marker for cell transformation and tumor growth. Additionally, γ-GT may accelerate tumor growth and increase the resistance of tumors to chemotherapeutic agents. Hanigan, M.H., et al., *Carcinogenesis*, 20(4):553-59 (1999). A number of hypotheses about γ-GT's physiological function have been proposed, but the best characterized *in vivo* function for γ-GT involves metabolism and transport of glutathione and its derivatives. *See, e.g.,* Hahn, R., et al., *Biochem. Biophys. Acta.*, 539:324-37 (1978); Griffith, O. W., et al., *Proc. Natl. Acad. Sci. USA,* 76:6319-22 (1979); Griffith, O. W., and Meister, A., *Proc. Natl. Acad. Sci. USA,* 77:3384-87 (1979). Although normally a degradative enzyme, γ-GT helps regulate glutathione metabolism by recycling cysteine (one of the three peptides in the glutathione molecule). Additionally, under certain conditions, γ-GT may facilitate the

biosynthesis of γ-glutamyl-cysteine (a glutathione precursor). Prezioso, J. A., et al., *Int. J. Radiation Oncology Biol. Phys.*, 30:373-81 (1994).

Mechanistically, γ-GT appears to function as a threonine protease. The mechanism of action for γ-GT involves an intermediate transition state where a γ-glutamyl is bound to a threonine residue located in the active site and on the lower molecular weight subunit of the enzyme. Various studies with rat, porcine, and human γ-GT enzymes and acivicin, a nonspecific irreversible γ-GT inhibitor, show that this threonine residue in the active site is esterified during inhibition. Therefore, it is likely that this esterification blocks the active site of γ-GT, rather than inactivating a critical catalytic amino acid residue.

A number of γ-GT inhibitors are known, in addition to acivicin. Most of these previously described γ-GT inhibitors are irreversible inhibitors. Such irreversible inhibitors include azaserine (O-diazoacetyl-L-serine), DON (6-diazo-5-oxo-norleucine), acivicin, and L-2-amino-4-fluorophosphono butanoic acid.

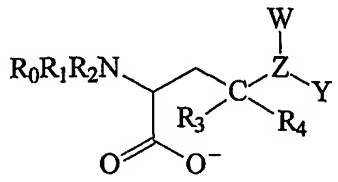
Two reversible γ-GT inhibitors have been described: L-γ-glutamyl-(*o*-Carboxy)Phenylhydrazine (commonly called "anthglutin") and γ-(L-γ-azaglutamyl)-L-cysteinyl-glycine. However, these reversible inhibitors have only moderate inhibitory activity, presumably because these compounds function as substrate analogs. Additionally, at least one study of anthglutin shows this compound is toxic to mice. Griffith, O. W., and Meister, A., *Proc. Natl. Acad. Sci. USA.* 76:268-72 (1979).

L-serine has been shown to inhibit γ-GT *in vitro* in the presence of boronate buffer, apparently as a serine-boronate complex that acts as a transition state analog. However, this serine-boronate complex requires the presence of a boronate buffer, which is not normally found under physiological conditions.

Therefore, a need exists for a reversible, effective, non-toxic γ-GT inhbitor that is stable under physiological conditions.

SUMMARY

This invention relates to compounds and methods of inhibiting γ-glutamyl transpeptidase (γ-GT) by contacting γ-GT with an effective inhibitory amount of a compound having the formula:



wherein R_0 is optional and may be H or lower alkyl;

R_1 is H or lower alkyl;

R_2 is H or lower alkyl;

R_3 is H or halogen;

R_4 is H or halogen;

Z is B or C;

W is =O when Z is C, or –OH when Z is B;

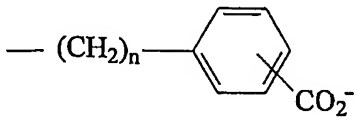
Y is –OH, lower alkyl, $-OPO_3$, $-(CH_2)_n-CO_2$, aryl, or $CR_5R_6R_7$; where

R_5 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_6 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_7 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl; and

where at least one of R_5, R_6, or R_7 is halogen, and n is 1-8, for example 1-5, or any of 1, 2, 3, 4, 5, 6, 7 or 8. In particular embodiments, the aryl is a benzyl, for example a substituted benzyl. One such example of a $-(CH_2)_n$-aryl where the aryl is a substituted benzyl is:



Particular embodiments employ compounds that are L-isomers, D-isomers, or mixtures of both L- and D-isomers. Exemplary compounds include L-2-amino-4-boronobutanoic acid (ABBA); 6,6,6-trifluoro-5-oxo-L-norleucine; and 6,6-difluoro-5-oxo-6-phosphono-L-norleucine.

These compounds may inhibit γ-GT associated with a cell, such as γ-GT located on a cell membrane, or γ-GT that is isolated apart from a cell. In some embodiments, the compound effectively inhibits γ-GT with an inhibition constant (K_i) of about 8000 nM or less. More particular embodiments employ a compound that inhibits γ-GT with a K_i value of less than about 1000 nM, such as an inhibition constant of about 17 nM. In certain embodiments, the compound is a specific inhibitor of γ-GT.

Certain embodiments include treating a condition or disease mediated by γ-GT, or a condition or disease where toxicity of glutathione adducts may be a concern. For example, some neoplasms may be treated by administering a therapeutic amount of a γ-GT inhibitor, particularly if the neoplasm expresses higher than normal levels of γ-GT. The neoplasm may inherently express γ-GT at a heightened level, or such heightened expression may be induced by some environmental stimulus, such as radiation or a chemical agent. As another example, the γ-GT inhibitor also may inhibit neoplastic resistance to chemotherapy and radiotherapy, or potentiate the effects of radiation and certain chemotherapeutic agents. Additionally, compounds may be administered to inhibit γ-GT to reduce the toxicity of some glutathione adducts or conjugates, for example to inhibit the development of renal toxicity induced by such agents.

In some embodiments, the neoplasm is a carcinoma. Particular carcinomas may, for example, arise in the kidney, lung, liver, prostate, breast, or thyroid. In particular embodiments, the carcinoma is selected from the group consisting of adenocarcinomas and other carcinomas, for example renal cell carcinoma, lung adenocarcinoma, pleura mesothelioma, stomach adenocarcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, ovarian surface epithelial carcinoma, uterine serous papillary carcinoma, mammary invasive ductal carcinoma, mammary invasive lobular carcinoma, thyroid follicular carcinoma, thyroid papillary carcinoma, and combinations thereof.

The compound may be a pharmaceutically acceptable acid addition, salt, ester, or prodrug; or comprise 6a pharmaceutically acceptable carrier, agent, counterion, adjuvant, or vehicle. The compound also may be administered in combination with another useful pharmaceutical agent or another γ-GT inhibitor.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A illustrates the transition state of γ-GT.

FIG. 1B illustrates a ternary complex formed from γ-GT and serine in the presence of borate buffer.

FIG. 1C illustrates the interaction of ABBA with the active site of γ-GT.

FIG. 2 illustrates the chemical interactions that occur during γ-GT inhibition by ABBA.

FIG. 3A is a Lineweaver-Burk plot of ABBA determined for varying substrate concentrations.

FIG. 3B is a replot of FIG. 2A with the slopes of the lines determined in FIG. 2A plotted against the inhibitor (ABBA) concentration.

DETAILED DESCRIPTION

Explanation of Terms

Unless otherwise noted, technical terms are used according to conventional usage.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an inhibitor" includes single or plural inhibitors and can be considered equivalent to the phrase "at least one inhibitor."

As used herein, the term "comprises" means "includes." For example, "comprising" A or B means includes A or B, or both.

In order to facilitate review of the various embodiments of the invention, the following definitions are provided:

An "animal" is a living multicellular vertebrate organism, a category which includes, for example, mammals, reptiles, arthropods, and birds.

"Adduct" refers to a compound produced by mixing two or more chemicals, including complexes that form when a chemical binds to a biological molecule.

The term "amino" refers to a chemical functionality $-NR_1R_2$, where R_1 and R_2 are independently hydrogen, alkyl, or aryl.

An "analog" is a molecule that differs in chemical structure from a parent compound. Examples include, but are not limited to: a homolog (which differs by an increment in the chemical structure, such as a difference in the length of an alkyl chain); a molecular fragment; a structure that differs by one or more functional groups; or a change in ionization. Structural analogs are often found using quantitative structure activity relationships (QSAR), with techniques such as those disclosed in *Remington: The Science and Practice of Pharmacology*, 19th Edition (1995), chapter 28. A derivative is a biologically active molecule derived from the base molecular structure. A mimetic is a biomolecule that mimics the activity of another biologically active molecule. Biologically active molecules can include both chemical structures and peptides that mimic the γ-GT inhibitory activities of the compounds disclosed herein.

The term "alkylamino" refers to a lower alkyl radical appended to an –NH radical.

The term "alkoxy" refers to a substituted or unsubstituted alkoxy, where an alkoxy has the structure –O–R, where R is a substituted or unsubstituted alkyl. In an unsubstituted alkoxy, the R is an unsubstituted alkyl. The term "substituted alkoxy" refers to a group having the structure –O–R, where R is alkyl which is substituted with a non-interfering substituent. "Lower alkoxy" refers to any alkoxy in which R is a lower alkyl. "Thioalkoxy" refers to –S–R, where R is substituted or unsubstituted alkyl.

The term "alkoxyalkyl" refers to an alkoxy group appended to a lower alkyl radical.

The term "alkyl" refers to a cyclic, branched, or straight chain alkyl group containing only carbon and hydrogen, which, unless otherwise described, contains one to twelve carbon atoms. This term is further exemplified by groups such as methyl, ethyl, n-propyl, isobutyl, t-butyl, pentyl, pivalyl, heptyl, adamantyl, and cyclopentyl. Alkyl groups can be unsubstituted or substituted with one or more substituents, for example halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "aryl" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g., phenyl, benzyl) or multiple condensed rings (e.g., naphthyl or anthryl), which can optionally be unsubstituted or substituted with, for example, halogen, alkyl, alkoxy, mercapto (-SH), alkylthio, trifluoromethyl, acyloxy, hydroxy, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

"Cancer" is a general term referring to diseases characterized by uncontrollable, abnormal growth of a cell. A cancer may be a malignant neoplasm. A "carcinoma" is a malignant neoplasm of epithelial tissue.

"Carbonyl containing group" refers to any substituent containing a carbon-oxygen double bond (C=O), including substituents based on –COR where R is an alkyl, lower alkyl, hydroxyl, or a secondary, tertiary, or quaternary amine. The term also encompasses oximes and hydrazones. Alternatively, "carbonyl containing group" refers to –R'COR groups wherein R is alkyl, lower alkyl, hydroxyl, or secondary, tertiary, or quaternary amine, and R' is alkylene, such as methylene (–CH_2–). Examples include –COOH, –CH_2COOH, –CH_2COOCH_3, –CH_2CONH_2, and –$CH_2CON(CH_3)_2$.

"Carboxyl" refers to the radical –COOH, and substituted carboxyl refers to –COR where R is alkyl, lower alkyl, or a carboxylic acid or ester.

"Conjugate" refers to an acid and a base that can convert to each other by the gain or loss of a proton.

The term "dialkylamino" refers to –N–R–R' wherein R and R' are independently selected from lower alkyl groups.

The term "dialkylaminoalkyl" refers to –N–R–R', which is appended to a lower alkyl radical, wherein R and R' are independently selected from lower alkyl groups.

The term "effective inhibitory amount" refers to an amount sufficient to inhibit the enzymatic activity of γ-GT to at least some degree.

The term "halogen" refers to the elements fluourine, bromine, chlorine, and iodine, and the term "halo" refers to fluoro, bromo, chloro and iodo substituents.

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biosynthesis of γ-glutamyl-cysteine (a glutathione precursor). Prezioso, J. A., et al., *Int. J. Radiation Oncology Biol. Phys.*, 30:373-81 (1994).

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A number of γ-GT inhibitors are known, in addition to acivicin. Most of these previously described γ-GT inhibitors are irreversible inhibitors. Such irreversible inhibitors include azaserine (O-diazoacetyl-L-serine), DON (6-diazo-5-oxo-norleucine), acivicin, and L-2-amino-4-fluorophosphono butanoic acid.

Two reversible γ-GT inhibitors have been described: L-γ-glutamyl-(*o*-Carboxy)Phenylhydrazine (commonly called "anthglutin") and γ-(L-γ-azaglutamyl)-L-cysteinyl-glycine. However, these reversible inhibitors have only moderate inhibitory activity, presumably because these compounds function as substrate analogs. Additionally, at least one study of anthglutin shows this compound is toxic to mice. Griffith, O. W., and Meister, A., *Proc. Natl. Acad. Sci. USA.* 76:268-72 (1979).

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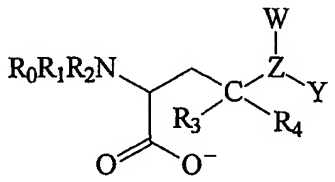
Therefore, a need exists for a reversible, effective, non-toxic γ-GT inhbitor that is stable under physiological conditions.

SUMMARY

This invention relates to compounds and methods of inhibiting γ-glutamyl transpeptidase (γ-GT) by contacting γ-GT with an effective inhibitory amount of a compound having the formula:



wherein R_0 is optional and may be H or lower alkyl;

R_1 is H or lower alkyl;

R_2 is H or lower alkyl;

R_3 is H or halogen;

R_4 is H or halogen;

Z is B or C;

W is =O when Z is C, or –OH when Z is B;

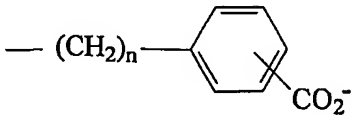
Y is –OH, lower alkyl, $-OPO_3$, $-(CH_2)_n-CO_2$, aryl, or $CR_5R_6R_7$; where

R_5 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_6 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_7 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl; and

where at least one of R_5, R_6, or R_7 is halogen, and n is 1-8, for example 1-5, or any of 1, 2, 3, 4, 5, 6, 7 or 8. In particular embodiments, the aryl is a benzyl, for example a substituted benzyl. One such example of a $-(CH_2)_n$-aryl where the aryl is a substituted benzyl is:



Particular embodiments employ compounds that are L-isomers, D-isomers, or mixtures of both L- and D-isomers. Exemplary compounds include L-2-amino-4-boronobutanoic acid (ABBA); 6,6,6-trifluoro-5-oxo-L-norleucine; and 6,6-difluoro-5-oxo-6-phosphono-L-norleucine.

These compounds may inhibit γ-GT associated with a cell, such as γ-GT located on a cell membrane, or γ-GT that is isolated apart from a cell. In some embodiments, the compound effectively inhibits γ-GT with an inhibition constant (K_i) of about 8000 nM or less. More particular embodiments employ a compound that inhibits γ-GT with a K_i value of less than about 1000 nM, such as an inhibition constant of about 17 nM. In certain embodiments, the compound is a specific inhibitor of γ-GT.

Certain embodiments include treating a condition or disease mediated by γ-GT, or a condition or disease where toxicity of glutathione adducts may be a concern. For example, some neoplasms may be treated by administering a therapeutic amount of a γ-GT inhibitor, particularly if the neoplasm expresses higher than normal levels of γ-GT. The neoplasm may inherently express γ-GT at a heightened level, or such heightened expression may be induced by some environmental stimulus, such as radiation or a chemical agent. As another example, the γ-GT inhibitor also may inhibit neoplastic resistance to chemotherapy and radiotherapy, or potentiate the effects of radiation and certain chemotherapeutic agents. Additionally, compounds may be administered to inhibit γ-GT to reduce the toxicity of some glutathione adducts or conjugates, for example to inhibit the development of renal toxicity induced by such agents.

In some embodiments, the neoplasm is a carcinoma. Particular carcinomas may, for example, arise in the kidney, lung, liver, prostate, breast, or thyroid. In particular embodiments, the carcinoma is selected from the group consisting of adenocarcinomas and other carcinomas, for example renal cell carcinoma, lung adenocarcinoma, pleura mesothelioma, stomach adenocarcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, ovarian surface epithelial carcinoma, uterine serous papillary carcinoma, mammary invasive ductal carcinoma, mammary invasive lobular carcinoma, thyroid follicular carcinoma, thyroid papillary carcinoma, and combinations thereof.

The compound may be a pharmaceutically acceptable acid addition, salt, ester, or prodrug; or comprise 6a pharmaceutically acceptable carrier, agent, counterion, adjuvant, or vehicle. The compound also may be administered in combination with another useful pharmaceutical agent or another γ-GT inhibitor.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A illustrates the transition state of γ-GT.

FIG. 1B illustrates a ternary complex formed from γ-GT and serine in the presence of borate buffer.

FIG. 1C illustrates the interaction of ABBA with the active site of γ-GT.

FIG. 2 illustrates the chemical interactions that occur during γ-GT inhibition by ABBA.

FIG. 3A is a Lineweaver-Burk plot of ABBA determined for varying substrate concentrations.

FIG. 3B is a replot of FIG. 2A with the slopes of the lines determined in FIG. 2A plotted against the inhibitor (ABBA) concentration.

DETAILED DESCRIPTION

Explanation of Terms

Unless otherwise noted, technical terms are used according to conventional usage.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an inhibitor" includes single or plural inhibitors and can be considered equivalent to the phrase "at least one inhibitor."

As used herein, the term "comprises" means "includes." For example, "comprising" A or B means includes A or B, or both.

In order to facilitate review of the various embodiments of the invention, the following definitions are provided:

An "animal" is a living multicellular vertebrate organism, a category which includes, for example, mammals, reptiles, arthropods, and birds.

"Adduct" refers to a compound produced by mixing two or more chemicals, including complexes that form when a chemical binds to a biological molecule.

The term "amino" refers to a chemical functionality $-NR_1R_2$, where R_1 and R_2 are independently hydrogen, alkyl, or aryl.

An "analog" is a molecule that differs in chemical structure from a parent compound. Examples include, but are not limited to: a homolog (which differs by an increment in the chemical structure, such as a difference in the length of an alkyl chain); a molecular fragment; a structure that differs by one or more functional groups; or a change in ionization. Structural analogs are often found using quantitative structure activity relationships (QSAR), with techniques such as those disclosed in *Remington: The Science and Practice of Pharmacology*, 19th Edition (1995), chapter 28. A derivative is a biologically active molecule derived from the base molecular structure. A mimetic is a biomolecule that mimics the activity of another biologically active molecule. Biologically active molecules can include both chemical structures and peptides that mimic the γ-GT inhibitory activities of the compounds disclosed herein.

The term "alkylamino" refers to a lower alkyl radical appended to an –NH radical.

The term "alkoxy" refers to a substituted or unsubstituted alkoxy, where an alkoxy has the structure –O–R, where R is a substituted or unsubstituted alkyl. In an unsubstituted alkoxy, the R is an unsubstituted alkyl. The term "substituted alkoxy" refers to a group having the structure –O–R, where R is alkyl which is substituted with a non-interfering substituent. "Lower alkoxy" refers to any alkoxy in which R is a lower alkyl. "Thioalkoxy" refers to –S–R, where R is substituted or unsubstituted alkyl.

The term "alkoxyalkyl" refers to an alkoxy group appended to a lower alkyl radical.

The term "alkyl" refers to a cyclic, branched, or straight chain alkyl group containing only carbon and hydrogen, which, unless otherwise described, contains one to twelve carbon atoms. This term is further exemplified by groups such as methyl, ethyl, n-propyl, isobutyl, t-butyl, pentyl, pivalyl, heptyl, adamantyl, and cyclopentyl. Alkyl groups can be unsubstituted or substituted with one or more substituents, for example halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "aryl" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g., phenyl, benzyl) or multiple condensed rings (e.g., naphthyl or anthryl), which can optionally be unsubstituted or substituted with, for example, halogen, alkyl, alkoxy, mercapto (-SH), alkylthio, trifluoromethyl, acyloxy, hydroxy, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

"Cancer" is a general term referring to diseases characterized by uncontrollable, abnormal growth of a cell. A cancer may be a malignant neoplasm. A "carcinoma" is a malignant neoplasm of epithelial tissue.

"Carbonyl containing group" refers to any substituent containing a carbon-oxygen double bond (C=O), including substituents based on –COR where R is an alkyl, lower alkyl, hydroxyl, or a secondary, tertiary, or quaternary amine. The term also encompasses oximes and hydrazones. Alternatively, "carbonyl containing group" refers to –R'COR groups wherein R is alkyl, lower alkyl, hydroxyl, or secondary, tertiary, or quaternary amine, and R' is alkylene, such as methylene (–CH_2–). Examples include –COOH, –CH_2COOH, –CH_2COOCH_3, –CH_2CONH_2, and –$CH_2CON(CH_3)_2$.

"Carboxyl" refers to the radical –COOH, and substituted carboxyl refers to –COR where R is alkyl, lower alkyl, or a carboxylic acid or ester.

"Conjugate" refers to an acid and a base that can convert to each other by the gain or loss of a proton.

The term "dialkylamino" refers to –N–R–R' wherein R and R' are independently selected from lower alkyl groups.

The term "dialkylaminoalkyl" refers to –N–R–R', which is appended to a lower alkyl radical, wherein R and R' are independently selected from lower alkyl groups.

The term "effective inhibitory amount" refers to an amount sufficient to inhibit the enzymatic activity of γ-GT to at least some degree.

The term "halogen" refers to the elements fluourine, bromine, chlorine, and iodine, and the term "halo" refers to fluoro, bromo, chloro and iodo substituents.

The term "heterocycle" (or "heterocyclic") refers to a monovalent saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g., benzyl, morpholino, pyridyl or furyl), or multiple condensed rings (e.g., naphthyl, quinolinyl, indolizinyl or benzo[b]thienyl), and having at least one heteroatom, (defined as N, O, P, or S) within the ring. A heterocycle can optionally be unsubstituted or substituted with, for example, halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality. Examples include, but are not limited to, aziridinyl, azetidinyl, pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, thiazolyl, oxazolyl, isoxazolyl, isothiazolyl, pyridinyl, pyrimidinyl, pyridazinyl, and pyrazinyl.

The term "(heterocyclic)alkyl" as used herein refers to a heterocyclic group appended to a lower alkyl radical including, but not limited to, pyrrolidinylmethyl and morpholinylmethyl.

"Hydroxyl" refers to –OH.

"Hydroxyalkyl" refers to –R–OH, wherein R is alkylene, especially lower alkylene (for example in methylene, ethylene, or propylene). A hydroxyalkyl group may be either linear or branched, such as 1-hydroxyisopropyl.

The term "labile" refers to a chemical bond that is capable of changing state or being destroyed by application of relatively low amounts of energy. Labile bonds exist for short periods of time, such as seconds, minutes, or even a few hours. Non-labile bonds exist for longer periods of time, such as about 5 hours, or indefinitely. Ionic bonds and hydrogen bonds are inherently labile. Most covalent bonds are non-labile, though some exceptions exist. A covalent bond between oxygen and boron, such as a borate ester, is just one example of a labile covalent bond. However, a carbon-boron covalent bond is considered to be non-labile.

The term "lower alkyl" refers to a cyclic, branched or straight chain monovalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), sec-butyl, n-pentyl, cyclopropylmethyl, i-amyl, n-amyl, n-pentyl, 1-methylbutyl, 2,2-dimethylbutyl, 2-methylpentyl, 2,2-dimethylpropyl, n-

hexyl. Lower alkyl groups can be unsubstituted or substituted. One specific example of a substituted alkyl is 1,1-dimethyl propyl.

The term "lower alkenyl" refers to a straight or branched chain alkyl radical containing from two to six carbon atoms and also having one carbon-carbon double bond including, but not limited to: vinyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, and 5-hexenyl.

A "mammal" includes both human and non-human mammals.

The term "=O" indicates a double-bonded oxygen moiety.

"Neoplasm" refers to a new and abnormal growth of tissue, which may be benign or malignant.

A "pharmaceutical agent," "pharmaceutical composition," or "drug" refers to a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject. The pharmaceutically acceptable salts of the compounds of this invention include, but are not limited to, those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide. These salts may be prepared by standard procedures, for example by reacting the free acid with a suitable organic or inorganic base. Any chemical compound recited in this specification may alternatively be administered as a pharmaceutically acceptable salt thereof.

The term "phenyl" refers to a phenyl group, which may be unsubstituted or substituted with a substituent selected from lower alkyl, alkoxy, thioalkoxy, hydroxy and halo.

The term "phenylalkyl" refers to a phenyl group appended to a lower alkyl radical including, but not limited to, benzyl, 4-hydroxybenzyl, 4-chlorobenzyl, and 1-naphthylmethyl.

The term "prodrug" refers to a compound that is converted within the body to a more active form that has medicinal or therapeutic effects.

The term "reversible" refers to a chemical reaction capable of proceeding in either of two directions, in contast to an "irreversible" chemical reaction that can proceed in only one direction. A reversible inhibitor of an enzyme is characterized by an ability to readily, even rapidly, dissociate from the enzyme-inhibitor complex. In contrast, an irreversible enzymatic inhibitor becomes tightly bound to the enzyme (either covalently or noncovalently) and dissociates slowly from the target enzyme. An irreversible inhibitor will usually form an adduct with the target enzyme, for example, via the formation of a non-labile covalent bond between the inhibitor and enzyme. An irreversible inhibitor that has bound to the target enzyme cannot be isolated apart from the target enzyme in any substantial amount after the inhibition interaction. If the enzyme is then digested by a protease (such as chymotripsin), one or more amino acids of the enzyme may remain attached to the irreversible inhibitor. In contrast, a reversible inhibitor usually will not form an adduct with the target enzyme and can be more readily isolated apart from the target enzyme after the inhibition interaction.

The term "stable compound" refers to a compound that is sufficiently stable to survive isolation to a useful degree of purity from a reaction mixture and formulation into a therapeutic dosage form suitable for administration.

The term "subject" includes both human and veterinary subjects, such as primates, canines, felines, and rodents.

The term "therapeutically effective amount" refers to an amount or dose sufficient to inhibit the enzymatic activity of γ-GT and is capable of relieving symptoms associated with γ-GT activation or expression.

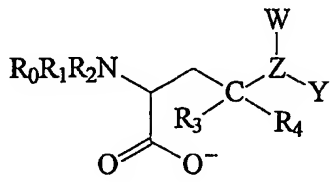
The term "thioalkoxyalkyl" refers to a thioalkoxy group appended to a lower alkyl radical.

Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (1985) and *The Condensed Chemical Dictionary* (1981).

All chemical compounds include both the L- and D-stereoisomers, as well as either the L- or D-stereoisomer, unless otherwise specified.

Inhibitors

The invention includes embodiments of a compound (according to Formula A below) and a method for inhibiting γ-glutamyl transpeptidase (γ-GT) by contacting γ-GT with an effective inhibitory amount of the compound. This compound is described by the formula:



Formula A

wherein R_0 is optional and may be H or lower alkyl;

R_1 is H or lower alkyl;

R_2 is H or lower alkyl;

R_3 is H or halogen;

R_4 is H or halogen;

Z is B or C;

W is =O when Z is C, or –OH when Z is B;

Y is –OH, lower alkyl, $-OPO_3$, $-(CH_2)_n-CO_2$, $-(CH_2)_n$-aryl, or $CR_5R_6R_7$; where

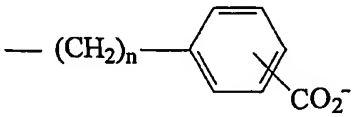
R_5 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_6 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_7 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl; and

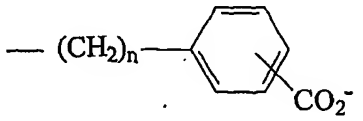
where at least one of R_5, R_6 or R_7 is halogen, and n is 1-8, for example 1-5, or any of 1, 2, 3, 4, 5, 6, 7 or 8.

In particular embodiments, the aryl is a benzyl, for example a substituted benzyl. One such example of a $-(CH_2)_n$-aryl where the aryl is a substituted benzyl is:



wherein CO_2^- is a carboxyl, but also could be a substituted carboxyl.

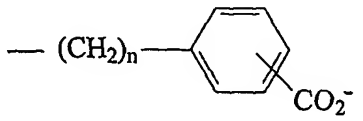
Specific embodiments employ compounds of Formula A wherein Z is B and W is –OH; Y is –OH, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl, such as



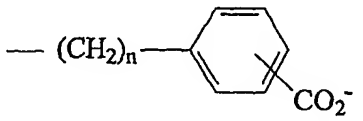
and, in some instances, wherein $R_1=R_2=H$.

In certain embodiments of Formula A, at least one of R_3, R_4, R_5, R_6 and R_7 is halogen, such as F. In more particular embodiments, at least one of R_3 and R_4 and at least one of R_5 and R_6 are halogen, such as F.

In other embodiments of Formula A, Z is C; R_3 or R_4 is H or halogen, such as F; W is =O; Y is $CR_5R_6R_7$; and at least one of R_5, R_6 or R_7 is halogen, such as F. In more particular embodiments, Z is C; W is =O; Y is $CR_5R_6R_7$; and at least two of R_5, R_6 and R_7 are F; or $R_1=R_2=H$, $R_3=R_4=F$, and $R_5=R_6=F$; or R_7 is $-(CH_2)_n-CO_2$, or



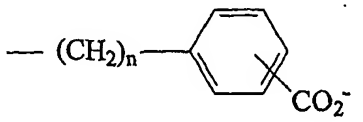
In still other embodiments of Formula A, $R_0=R_1=R_2=R_4=H$; Z is B, W is –OH, and Y is $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl, such as



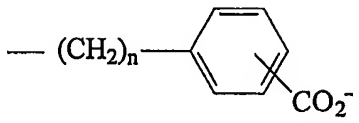
In still other embodiments of Formula A, $R_0=R_1=R_2=R_3=R_4=H$; Z is C; W is =O; Y is $CR_5R_6R_7$; R_5 is $-PO_3H_2$; R_6 is F; and R_7 is F.

In still other embodiments of Formula A, $R_0=R_1=R_2=R_3=R_4=H$; Z is B; and both W and Y are –OH.

Other embodiments employ compounds of Formula A wherein $R_0=R_1=R_2=H$; $R_3=R_4=F$; Z is C, W is =O; and Y is $CR_5R_6R_7$ wherein $R_5=R_6=R_7=F$, or $R_5=R_6=F$ and R_7 is $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl, such as



In more particular embodiments, R_5=R_6=R_7=F; or R_5=R_6=F, and R_7 is –$(CH_2)_n$-CO_2, or –$(CH_2)_n$-aryl, such as



In other particular embodiments, R7 is

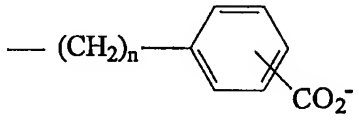
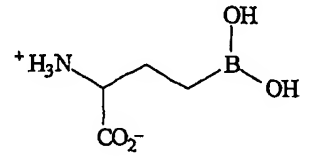
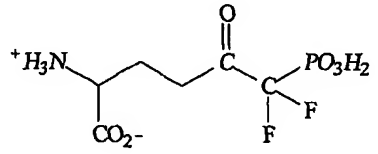
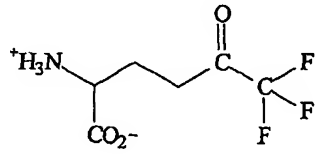
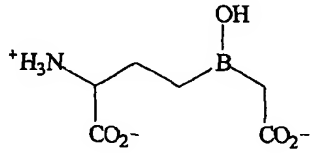
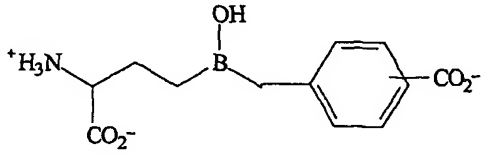
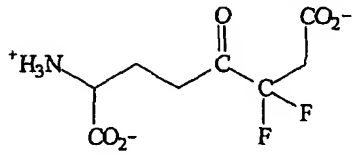
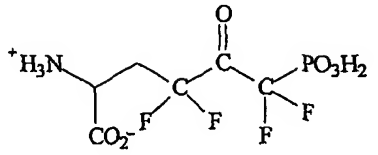
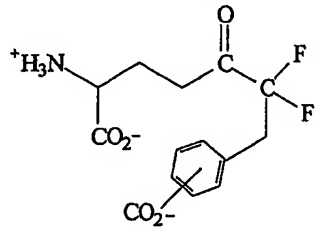


Table 1 illustrates some particular known compounds of Formula A.

Table 1—Representative γ-Glutamyl Transpeptidase Inhibitors

L-2-amino-4-boronobutanoic acid (ABBA)	
6,6-difluoro-5-oxo-6-phosphono-norleucine	
6,6,6-trifluoro-5-oxo-norleucine	
2-amino-5-borono acid-di-heptanoic acid	
2-amino-5-borono acid-6-benzoic acid hexaoanoic acid	
2-amino-5-oxo-6-difloro-di-octanoic acid	
4,6-tetrafluoro-5-oxo-6-phosphono norleucine	

2-amino-5-oxo-6-difluoro-7-benzoic heptanoic acid	

All of these compounds may be either L- or D-isomers, though particular embodiments employ compounds that are L-isomers, D-isomers, or mixtures of both L- and D-isomers.

In particular embodiments, the compound is a reversible inhibitor of γ-GT. For example, ABBA is a reversible and effective γ-GT inhibitor (see Example 1 below).

The mechanism of inhibition involves a compound that is a structural analog of glutamic acid or glutamine and mimics the transition state of the enzyme. The γ-GT active site contains a nucleophile, such as the hydroxyl of a threonine (e.g., Thr^{391} in the *E. coli* γ-GT), which interacts and bonds with the compound.

FIG. 1A illustrates the transition state of γ-GT. FIG. 1B illustrates a ternary complex formed by the interaction of serine and γ-GT in the presence of borate buffer. This serine-borate complex can weakly inhibit γ-GT at millimolar concentrations. Tate, S. S. and Meister, A., *Proc. Natl. Acad. Sci. USA* 75(10):4806-09 (1978). Serine in a borate buffer will rarely bond with borate molecules. However, if γ-GT is added, the γ-GT may independently interact with serine and borate, and the serine and borate may be brought together in the γ-GT active site to form a serine-borate complex. As illustrated in FIG. 1B, this complex forms a labile covalent bond between the boron of the boronic acid and the hydroxyl oxygen of the serine. This labile bond is considered to be a serine-borate oxygen ester linkage. The γ-GT itself stabilizes this serine-borate complex in the active site. However, if either the serine or borate dissociates from the active site, the serine-borate complex will fall apart because the labile serine-borate oxygen ester linkage is not normally strong enough to hold the serine and borate together.

In contrast, a boronic compound according to Formula A (i.e., where Z is boron) has a non-labile bond, since a carbon takes the place of the serine hydroxyl oxygen in the labile serine-borate oxygen ester linkage. This carbon-boron bond is considered to be non-labile. Other compounds according to Formula A (i.e., where Z is carbon) have a non-labile carbon-carbon bond. Thus, a compound according to Formula A is a more stable inhibitor of γ-GT, compared to the serine-borate complex with the enzyme, and will demonstrate more potent inhibition of γ-GT compared to the ternary complex illustrated in FIG. 1B. For example, as seen in FIG. 1C, ABBA has a non-labile methylene boronate linkage, rather than the labile boron-oxygen bond between the serine and borate portions of the ternary complex illustrated in FIG. 1B.

The chemistry of the interaction between ABBA and γ-GT is further illustrated in FIG. 2. Other compounds according to Formula A form similar bonds with the active site nucleophile, though the nucleophile bonds to a carbonyl carbon rather than boron. For example, if 6,6-difluoro-5-oxo-6-phosphono-L-norleucine was used as the γ-GT inhibitor, the nucleophile (e.g., the hydroxyl of the threonine in the active site) would bond with the carbonyl carbon at the fifth position of the compound, releasing one of the two bonds between the oxygen and the carbon.

Some compounds according to Formula A inhibit γ-GT at nanomolar concentrations. Certain embodiments employ a compound according to Formula A that inhibits γ-GT at a K_i value less than about 8000 nM. Other embodiments provide even more effective inhibition of γ-GT, such as inhibiting γ-GT at a K_i value less than about 5000 nM, less than about 2500 nM, less than about 1000 nM, less than about 500 nM, less than about 250 nM, less than about 100 nM, less than about 50 nM, or less than about 25 nM. As just one example, ABBA inhibits γ-GT with a K_i value of about 17 nM, as demonstrated in Example 1.

Some of the compounds listed in Table 1 are ketones, which typically undergo nucleophilic addition during chemical reaction. Thus, these compounds may interact with a nucleophile in the γ-GT active site (e.g., a hydroxyl of threonine) through the carbonyl-carbon. Electron-withdrawing substituents, such as fluorine, added to the carbonyl-carbon will enhance this nucleophilic attraction. Additionally, a ketone according to Formula A will have a tetrahedral geometry around the

carbonyl group, if the compound is hydrated. Such a tetrahedral geometry provides a structural basis for inhibiting γ-GT, in addition to the functional nature of the carbonyl group as a site for nucleophilic addition.

Methods of Use and Treatment

Certain embodiments of the invention relate to inhibiting the activity of γ-GT that is located within a cell, such as on a cell membrane, including (but not limited to) *in situ* studies of γ-GT activity, or *in vivo* assays using cell cultures. In particular embodiments, the cell may be immortalized, such as a cell isolated from a cell culture arising from a neoplasm. Additionally, the cell may be of a particular tissue type, such as a kidney cell or white blood cell.

Alternative embodiments encompass inhibiting the activity of γ-GT that is not associated with a cell, such as measuring the enzyme kinetics of γ-GT *in vitro*. In certain instances, the γ-GT will be isolated away from other biomolecules. In alternative embodiments, the isolated γ-GT may be inhibited in a liposome or similar environment.

Other embodiments of the invention employ methods of inhibiting γ-GT with a compound according to Formula A, for example, in treating a condition or disease. In such embodiments, a γ-GT inhibitor according to Formula A is administered in a therapeutically effective amount to a subject. Such embodiments are particularly useful in conditions or diseases that are mediated by γ-GT, or conditions or diseases where toxicity of glutathione adducts or conjugates may be a concern.

Some neoplasms, for example, malignant neoplasms, may be treated by administering a therapeutic amount of a compound according to Formula A. Some neoplasms express γ-GT, and may express γ-GT at heightened levels compared to other tissues. This heightened expression of γ-GT may be a natural expression of the neoplasm, or may be induced by some environmental stimulus, such as radiation or chemical agents. In some neoplasms, the γ-GT adopts an intracellular orientation, rather than the typical extracellular orientation, thus enhancing the ability of the neoplastic cells to recycle cysteine and glutathione. Therefore, in certain embodiments, the compound according to Formula A is transported into the cell to contact the γ-GT intracellularly.

The γ-GT inhibitor compound may inhibit growth of the neoplasm if administered in therapeutically effective amounts. Tumors to be treated with the γ-GT inhibitor may be selected, for example, by determining whether the tumor overexpresses γ-GT, including using the method shown in Hanigan, M. H., *Human Pathology*, 30(3):300-05 (1999). Examples of γ-GT overexpressing tumors include lung adenocarcinoma, mammary invasive ductal carcinoma, hepatocellular carcinoma, and prostate adenocarcinoma. Individual subjects can be selected for treatment by the inhibitors disclosed herein based on pathological diagnosis that the individual has a tumor type that usually overexpresses γ-GT. Alternatively, γ-GT levels of individual tumors can be measured to determine whether γ-GT levels are increased in a particular tumor, in which case treatment with the inhibitor may be initiated.

In alternative embodiments, the compound is administered in association with another anti-neoplastic treatment. Exemplary anti-neoplastic treatments include anti-neoplastic chemotherapy or radiotherapy. Under such treatments, the neoplasm may become resistant to the chemotherapy and/or radiotherapy via expression of γ-GT. Thus, the γ-GT inhibitor also may inhibit neoplastic resistance to chemotherapy and radiotherapy.

In other embodiments, the γ-GT inhibitor potentiates the effects of radiation and certain drugs. Glutathione may protect cells against radiation and certain drugs, such as cisplatin. By inhibiting γ-GT (thus interfering with glutathione metabolism), the compounds according to Formula A may beneficially potentiate the effects of radiation and other agents on cells.

Renal toxicity may result from exposure to environmental, pharmacological, or other xenobiotic agents that form glutathione adducts or conjugates *in vivo*. These adducts or conjugates may be subsequently degraded in the kidney by γ-GT. For example, in some courses of anti-neoplastic chemotherapy, administered chemotherapeutic agents (e.g., cisplatin, doxorubicin) may form adducts or conjugates with intracellular or extracellular glutathione. Cells of the kidney may metabolize glutathione conjugates or adducts to recover cysteine, and metabolism of glutathione adducts or conjugates can lead to chemical species, such as mercapturic acids, that are more reactive and exhibit greater toxicity than the adduct or conjugate

itself. Thus, the method includes embodiments where inhibiting γ-GT using a compound according to Formula A decreases metabolism of glutathione conjugates or adducts and, in turn, reduces renal toxicity.

In some embodiments described above, the neoplasm is a carcinoma. Particular carcinomas may arise in certain organs or tissues, such as the kidney, lung, liver, prostate, breast, or thyroid. In particular embodiments, the carcinoma is selected from the group consisting of adenocarcinomas and other carcinomas, for example renal cell carcinoma, lung adenocarcinoma, stomach adenocarcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, ovarian surface epithelial carcinoma, uterine serous papillary carcinoma, mammary invasive ductal carcinoma, mammary invasive lobular carcinoma, thyroid follicular carcinoma, thyroid papillary carcinoma, and combinations thereof.

In particular embodiments, neoplasms exhibiting increased γ-GT enzyme are selected for treatment. Overexpression of γ-GT can be determined by immunocytochemistry, *in situ* hybridization, RT-PCR, or *in situ* PCR. For example, γ-GT expression can be detected by immunostaining with GGT129, an affinity-produced polyclonal antibody directed against a 20-amino-acid peptide corresponding to the C-terminus of the heavy subunit of human γ-GT. Hanigan, M., et al., *Human Pathology*, 30(3):300-05 (1999). Additionally, γ-GT activity can be assayed biochemically using γ-glutamyl-p-nitroanilide as a substrate and measuring amounts of the resulting p-nitroanilide using spectrophotometry. *See, e.g.*, Wright, E. C., et al., *J. Inher. Metab. Dis.*, 2-7 (1979); *see also* Example 1 below.

In any embodiment, the compound may be a pharmaceutically acceptable acid addition, salt, ester, or prodrug. Additionally, the compound may include a pharmaceutically acceptable carrier, agent, counterion, adjuvant, or vehicle. In some embodiments, the compound may be orally bioavailable and/or stable under physiological conditions, such as physiological pH.

Some embodiments include administering another useful pharmaceutical agent, such as a pharmaceutical agent useful for treating the condition or disease. Since glutathione represents a defensive mechanism of a neoplastic cell against radiation or chemotherapy, some embodiments include administering a γ-GT inhibitor according to Formula A in combination with another inhibitor of

glutathione synthesis (e.g., γ-glutamyl cysteine synthetase, glutamine synthetase). For example, the γ-GT inhibitor can be used in combination with buthionine sulfoximine (BSO), an inhibitor of γ-glutamyl cysteine synthetase. Particular embodiments employ another γ-GT inhibitor, such as a currently known γ-GT inhibitor; another γ-GT inhibitor according to Formula A; or another antineoplastic agent specifically used to treat a particular carcinoma (e.g., taxol or vinblastine in the treatment of a lung carcinoma, tamoxifen or doxorubicin in the treatment of breast carcinoma, or methotrexate in the treatment of ovarian carcinoma).

While the present invention is described in connection with several embodiments, the scope of the present invention is not intended to be limited to any particular embodiment. Instead, the descriptions and examples disclosed are intended to cover all alternatives, modifications, and equivalents that may be included within the spirit and scope of the invention as defined by the claims.

EXAMPLES

Example 1—γ-GT Inhibition with ABBA

L-2-amino-4-borono butanoic acid (ABBA), an amino acid analog, was demonstrated to be an effective γ-GT inhibitor.

FIG. 1A illustrates the proposed transition state of γ-GT. The enzyme binding site contains a positively charged group interacting with the α-carboxyl, an anionic group interacting with the α-amino, and an N-terminal threonine residue (Thr^{391} in the *E. coli* γ-GT) forming a tetrahedral adduct with the γ-carbonyl. As shown in FIG. 1B, the proposed mechanism of inhibition for a serine-borate buffer mixture involves the formation of a ternary enzyme-L-serine-borate complex that functions as a transition state analog. The labile serine-boronate ester linkage is stabilized by the interaction with the enzyme. In contrast, as shown in FIG. 1C, ABBA replaces this labile serine-boronate ester linkage with a non-labile methylene boronate linkage, resulting in more potent inhibition of γ-GT. Since ABBA contains this non-labile carbon-boron bond, it can reversibly form a complex with γ-GT via the tetrahedral boronate group, which presumably links directly to the threonine oxygen in the active site. This interaction mimics the transition state complex formed by the enzyme.

ABBA was obtained from Paradigm Organics (Raleigh, NC). γ-GT, derived from bovine kidney, was obtained from Sigma (St. Louis, MO) and was assayed using γ-glutamyl-p-nitroanalide (Sigma) as the substrate and glycl-glycine as the acceptor. *See* Furakawa, M., et al., *J. Biochem.*, 93:839-46 (1983); Gruber, H., et al., *J. Clin. Chem. Clin. Biochem.*, 15:565-73 (1977). Kinetic measurements were made by following the time-dependent increase in absorbance due to hydrolyzed p-nitroanalide at 410 nm. Measurements were made at 37°C on a Beckman DU 640 Spectrophotometer. The buffer consisted of 0.1 M HEPES (pH 8.0), 0.02 M glycyl-glycine, γ-glutamyl-p-nitroanalide concentrations from 0.5 to 4 mM, 0.2 μg enzyme, and varying concentrations of inhibitor.

FIG. 3A is a Lineweaver-Burke plot with an apparent Michaelis constant of 1.0 nM for the γ-glutamyl-p-nitroanalide and shows an inhibition constant of 17 nM. This plot was determined for γ-glutamyl-p-nitroanalide concentrations of 0.5, 1, 2,

or 4 mM in the presence of 20 mM glycyl-glycine acceptor in 100 mM HEPES (pH 8.0) buffer. $1/v_i$ is given in arbitrary units, and 1/[S] concentration in mM^{-1}. As shown in FIG. 3B (a replot of FIG. 3A), these kinetic studies demonstrated that ABBA acts by simple competitive inhibition with the γ-glutamyl-p-nitroanalide substrate.

ABBA is a more effective inhibitor compared to known reversible γ-GT inhibitors. For example, the L-isomer of anthglutin has a reported inhibition constant of 8.2 μM (8200 nM), while the D-isomer of anthglutin has a reported inhibition constant of 22.5 μM (22,500 nM).

Example 2—ABBA Inhibition of Cells Expressing γ-GT

ABBA significantly inhibited growth of mammalian cells.

Two rat liver cell lines were obtained from Dr. Michael Meredith (Oregon Health Sciences University, Portland, OR): nontumorigenic ARL-$15C_1$ and tumorigenic ARL-$16T_2$. ARL-$15C_1$ contained low levels of γ-GT, whereas ARL-$16T_2$ contained high levels of γ-GT. Both cell lines were cultured using Williams Media E (WME), with 10% fetal calf serum. Studies were conducted by inoculating 100 mm tissue culture dishes (Falcon) with 5×10^4 cells per plate. Each plate contained 10 mL WME. The plates were exposed to ABBA after 24 hours.

For studies in low cysteine media, the WME was aspirated from the plates after the initial 24 hour growth period and replaced with a modified WME that contained no cysteine or low cysteine (0.05 mM), plus 10% fetal calf serum. Half the cells were also treated with 10 μM ABBA.

Cell growth was measured at 24-hour intervals. Harvesting cells included rinsing the plates two times with phosphate buffered saline, adding 1.5 mL of 0.25% trypsin (Gibco) to cleave the cells from the plate, scraping the plate, and adding back 5 mL of WME plus 10% fetal calf serum to inactivate the trypsin. Cells were counted using a Beckman Coulter Multisizer II (Fullerton, CA).

Inoculation with 1 mM ABBA demonstrated significant reduction in cell counts in both cell lines. For example, at 48 hours after inoculation, the percent growth inhibition for the two cell lines was 23.0 ± 3.9% for ARL-$15C_1$, and 17.9 ± 5.5% for ARL-$16T_2$.

Example 3—Pharmaceutical Compositions and Modes of Administration

Various delivery systems for administering γ-GT inhibitors are known, and include, for example, encapsulation in liposomes, microparticles, microcapsules, and receptor-mediated endocytosis. *See, e.g.*, Wu and Wu, *J. Biol. Chem.*, 262:4429-32 (1987). However, an attractive feature of γ-GT as a drug target is its localization on the outer surface of most cells. Therefore, any γ-GT inhibitor that enters the bloodstream should eventually come into contact with a γ-GT molecule; the γ-GT inhibitor is not required to cross the cell membrane in order to interact with a γ-GT molecule in a normal extracellular orientation. However, γ-GT may adopt an intracellular orientation and, in this case, the γ-GT inhibitor usually must enter the cell in order to inhibit the γ-GT.

Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment, for example, by local infusion during surgery; topical application, such as in conjunction with a wound dressing after surgery; by injection; through a catheter; or by a suppository or an implant, such as a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

The use of liposomes as a delivery vehicle is one delivery method. The liposomes fuse with the target site and deliver the contents of the lumen intracellularly. The liposomes are maintained in contact with the target cells for a sufficient time for fusion to occur, using various means to maintain contact, such as isolation and binding agents. Liposomes may be prepared with purified proteins or

peptides that mediate fusion of membranes, such as Sendai virus or influenza virus. The lipids may be any useful combination of known liposome forming lipids, including cationic lipids, such as phosphatidylcholine. Other potential lipids include neutral lipids, such as cholesterol, phosphatidyl serine, phosphatidyl glycerol, and the like. For preparing the liposomes, the procedure described by Kato et al., *J. Biol. Chem.*, 266:3361-64 (1991), may be used.

Certain pharmaceutical compositions include a therapeutically effective amount of the γ-GT inhibitor and a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile, and the formulation can suit the mode of administration. The composition can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate.

In a particular embodiment, the composition is formulated in accordance with routine procedures, such as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition also may include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule, indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline.

The compositions may be administered intravenously in any conventional medium for intravenous injection, such as an aqueous saline medium, or in blood plasma medium. The medium also may contain conventional pharmaceutical

adjunct materials such as, for example: pharmaceutically acceptable salts to adjust the osmotic pressure; lipid carriers, such as cyclodextrins; proteins, such as serum albumin; hydrophilic agents, such as methyl cellulose; detergents; buffers; preservatives; and the like. A more complete explanation of parenteral pharmaceutical carriers can be found in *Remington: The Science and Practice of Pharmacy* (19th ed., 1995) in chapter 95.

Embodiments of other pharmaceutical compositions can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions. In particular embodiments, the compositions are in the form of a unit dose in solid, semi-solid, or liquid dosage form, such as tablets, pills, powders, liquid solutions, or suspensions.

The compositions can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, and procaine.

The amount of γ-GT that will be effective in the treatment of a particular disease or condition will depend on the nature of the disease or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or condition, and should be decided according to the judgment of the practitioner and each subject's or patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro, in vivo,* or animal model test systems.

The pharmaceutical compositions may be stored or carried within a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological

products. The notice also may reflect approval by the agency of manufacture, use, or sale for human administration.

The specific dose level and frequency of dosage for any particular subject may be varied and will depend on a variety of factors, including the activity of the specific compound; the metabolic stability and length of action of that compound; the age, body weight, general health, sex, diet of the subject; the mode and time of administration; rate of excretion; drug combination; and severity of the condition of the subject undergoing therapy.

Example 4—Effect of γ-GT Inhibitors in Combination with Other Antineoplastic Therapies

The pharmaceutical compositions or methods of treatment may be administered in combination with other therapeutic treatments, such as other antineoplastic therapies. For example, the γ-GT inhibitors may be administered in combination with effective doses of other antineoplastic compounds, other γ-GT inhibitors, or inhibitors of glutathione biosynthesis generally (e.g., BSO). The γ-GT inhibitors also may be administered in combination with radiation therapy, chemotherapy, or surgical therapy. The term "administration" includes both concurrent and sequential administration of the active agents, and the γ-GT inhibitors may be administered in combination with any composition (such as a chemotherapeutic agent), radiation, or surgical procedure for the treatment of neoplasms.

Examples of compositions that may be administered in combination with the γ-GT inhibitors include (but are not limited to) antimetabolites, plant alkaloids, topoisomerase inhibitors, alkylating agents, and antitumor antibiotics, such as: fluoracil, gemcitabine, methotrexate, carboplatin, cisplatin, paclitaxel, vinorelbine, doxorubicin, etoposide, mitoxantrone, cyclophosphamide, ifosfamide, mitomycin C, and dacarbazine.

Examples of radiotherapies that may be employed in combination with the γ-GT inhibitors include: systemic administration of iodine-131; systemic administration of strontium-89; directed administration of ionizing radiation, such as x-rays or gamma rays, using teletherapy or brachytherapy; particle beam therapy

using neutron or proton beams. A more complete explanation of antineoplastic therapies can be found in *Harrison's Principals of Internal Medicine* (14th ed., 1998) in chapter 86.

Antineoplastic agents, which are being used in combination with γ-GT inhibitor therapy (e.g., ABBA therapy), can be clinically used in accepted clinical protocols, for example, those given in the *Physician's Desk Reference* (1999 Ed.) and in Goodman and Gilman, *The Pharmacological Basis of Therapeutics,* Section XIII, *Chemotherapy of Neoplastic Disease* by Calabresi and Parks, pp. 1240-1306 (7th Ed. 1985, Macmilian Publishing Co, New York).

Example 5—Assays for γ-GT Inhibitory Activity

Methods of selecting γ-GT inhibitors useful for treating cancer, such as for use in a multidrug chemotherapeutic regimen, may be determined by *in vitro* pharmacosensitivity tests. Such tests employ well known methods of assaying *in vitro* activity for selecting compounds appropriate for *in vivo* uses. Examples of known assaying methods include the methods disclosed in U.S. Pat. Nos. 5,736,129 and 5,270,172.

Example 6—Reducing Renal Toxicity of Chemotherapeutic Agents

Griffith and Meister (*Proc. Natl. Acad. Sci. USA*, 76(1):268-72 (1979)) have administered a γ-GT inhibitor, γ-glutamyl(o-caboxy)phenylhdrazide, to mice (0.5 mmol/kg) and found that this level of adminstration blocked the metabolism of labeled γ-glutamyl-L-a-amino[14C]butyrate. Based on its much stronger inhibition constant determined *in vitro*, administration of 0.01-0.1 mM/kg of ABBA is sufficient to block renal γ-GT activity for a period of several hours. Administration of ABBA simultaeously with a chemotherapeutic agent, such as cisplatin, would inhibit breakdown of the chemotherapeutic agent in the kidney, promote excretion of the cisplatin-glutathione adduct, and, therefore, reduce renal toxicity.

Example 7—Reducing Renal Toxicity of Chemotherapeutic Agents

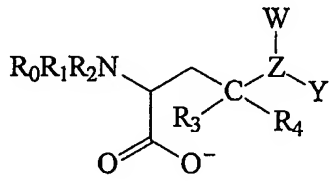
Under some conditions, degradation of glutamine or glutamine-containing molecules by γ-GT has been found to result in excessive production of ammonia.

See, e.g., Takahashi, S., et al., *J. Cellular Physiol.*, 125:107-114 (1985). Administration of a γ-GT inhibitior, such as ABBA, effectively reduces or stops this excess ammonia formation.

In view of the many possible embodiments to which the principles of this invention may be applied, it should be recognized that the examples are only illustrative examples of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is in accord with the scope and spirit of the following claims.

WE CLAIM:

1. A method of inhibiting γ-glutamyl transpeptidase, comprising contacting γ-glutamyl transpeptidase with an effective inhibitory amount of a compound having the formula:



wherein R_0 is optional and may be H or lower alkyl;

R_1 is H or lower alkyl;

R_2 is H or lower alkyl;

R_3 is H or halogen;

R_4 is H or halogen;

Z is B or C;

W is =O when Z is C, or –OH when Z is B;

Y is –OH, lower alkyl, $-OPO_3$, $-(CH_2)_n-CO_2$, $-(CH_2)_n$-aryl, or $CR_5R_6R_7$; where

R_5 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_6 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

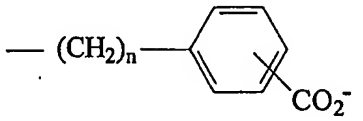
R_7 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl; and

where at least one of R_5, R_6 or R_7 is halogen, and n is 1-8.

2. The method according to claim 1, wherein the aryl is benzyl.

3. The method according to claim 2, wherein the benzyl is a substituted benzyl.

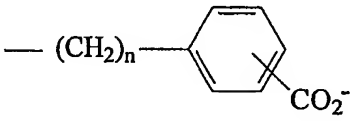
4. The method according to claim 3, wherein the substituted benzyl is



5. The method according to claim 1, wherein Z is B and both W and Y are –OH.

6. The method according to claim 1, wherein Z is B and W is –OH.

7. The method according to claim 6, wherein Y is $-(CH_2)_n-CO_2$, or



8. The method according to claim 7, wherein $R_1=R_2=H$.

9. The method according to claim 1, wherein at least one of R_3, R_4, R_5, R_6 and R_7 is F.

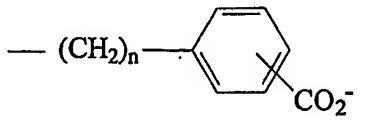
10. The method according to claim 9, wherein at least one of R_3 and R_4, and at least one of R_5 and R_6, are F.

11. The method according to claim 1, wherein Z is C, R_3 or R_4 is F, and Y is $CR_5R_6R_7$, wherein at least one of R_5, R_6 or R_7 is F.

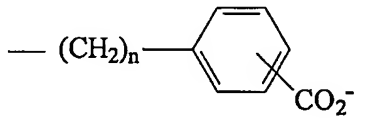
12. The method according to claim 11, wherein at least two of R_5, R_6 and R_7 are F.

13. The method according to claim 12, wherein $R_1=R_2=H$, $R_3=R_4=F$, and $R_5=R_6=F$.

14. The method according to claim 13, wherein R_7 is $-(CH_2)_n-CO_2$, or

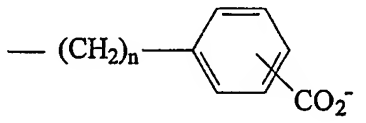


15. The method according to claim 1, wherein $R_0=R_1=R_2=R_3=R_4=H$; Z is B, W is –OH, and Y is $-(CH_2)_n-CO_2$, or



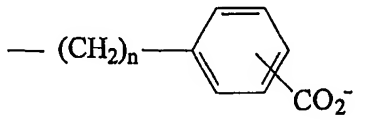
16. The method according to claim 1, wherein $R_0=R_1=R_2=R_3=R_4=H$; Z is C, W is =O; and Y is $-OPO_3$.

17. The method according to claim 1, wherein $R_0=R_1=R_2=H$; $R_3=R_4=F$; Z is C, W is =O; and Y is $CR_5R_6R_7$, wherein $R_5=R_6=R_7=F$, or $R_5=R_6=F$ and R_7 is $-(CH_2)_n-CO_2$, or

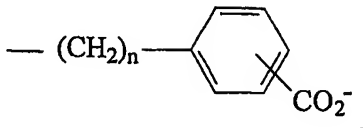


18. The method according to claim 17, wherein $R_5=R_6=R_7=F$.

19. The method according to claim 17, wherein $R_5=R_6=F$, and R_7 is $-(CH_2)_n-CO_2$, or



20. The method according to claim 17, wherein R_7 is



21. The method according to claim 1, wherein the compound is an L-isomer.

22. The method according to claim 1, wherein the method comprises a method of treating a disease by administering a therapeutically effective amount of the compound of claim 1 to inhibit γ-glutamyl transpeptidase.

23. The method according to claim 22, wherein the disease comprises a neoplasm.

24. The method according to claim 23, wherein the neoplasm is a malignant neoplasm.

25. The method according to claim 23, wherein the method further comprises administering the compound in association with another anti-neoplastic treatment that increases sensitivity of the neoplasm to the γ-glutamyl transpeptidase inhibitor.

26. The method according to claim 25, wherein the other anti-neoplastic treatment comprises anti-neoplastic chemotherapy or radiotherapy.

27. The method according to claim 26, wherein the compound inhibits the resistance of the neoplasm to the anti-neoplastic chemotherapy or radiation.

28. The method according to claim 26, wherein the other anti-neoplastic therapy comprises administering an agent that forms a glutathione adduct or conjugate *in vivo*.

29. The method according to claim 28, wherein the glutathione adduct or conjugate has a toxicity when metabolized by γ-glutamyl transpeptidase and the compound reduces the toxicity.

30. The method according to claim 23, wherein the neoplasm expresses higher than normal amounts of γ-glutamyl transpeptidase.

31. The method according to claim 23, wherein the neoplasm is a carcinoma.

32. The method according to claim 31, wherein the carcinoma arises in the kidney, lung, liver, prostate, breast, or thyroid.

33. The method according to claim 31, wherein the carcinoma is an adenocarcinoma.

34. The method according to claim 31, wherein the carcinoma is selected from the group consisting of: renal cell carcinoma, lung adenocarcinoma, stomach adenocarcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, ovarian surface epithelial carcinoma, uterine serous papillary carcinoma, mammary invasive ductal carcinoma, mammary invasive lobular carcinoma, thyroid follicular carcinoma, thyroid papillary carcinoma, and combinations thereof.

35. The method according to claim 1, wherein the compound comprises a pharmaceutically acceptable acid addition, salt, ester, or prodrug.

36. The method according to claim 1, wherein the compound comprises a pharmaceutically acceptable carrier, agent, counterion, adjuvant, or vehicle.

37. The method according to claim 22, further comprising administering another pharmaceutical agent useful for treating the disease.

38. The method according to claim 37, wherein the pharmaceutical agent useful for treating the disease comprises another γ-glutamyl transpeptidase inhibitor.

39. The method according to claim 1, wherein the compound is orally bioavailable.

40. The method according to claim 1, wherein the compound is stable at physiological pH.

41. The method according to claim 1, wherein the γ-glutamyl transpeptidase is located on a cell membrane.

42. The method according to claim 41 wherein the cell is immortalized.

43. The method according to claim 41 wherein the cell is a neoplastic cell.

44. The method according to claim 41 wherein the cell is a kidney cell.

45. The use of a compound according to claim 1 for use in the treatment of a condition mediated by γ-glutamyl transpeptidase.

46. The use of the compound according to claim 45, wherein the condition is a disease.

47. The use of the compound according to claim 46, wherein the disease is a neoplasm.

48. The use of the compound according to claim 47, wherein the neoplasm is a carcinoma.

49. The method according to claim 1, wherein the halogen is fluorine.

50. The method according to claim 1, wherein the compound comprises a reversible inhibitor.

51. The method according to claim 1, wherein the compound is an analog of glutamic acid or glutamine.

52. The method according to claim 1, wherein the compound forms a chemical bond with a nucleophile in the active site of γ-glutamyl transpeptidase.

53. The method according to claim 52 wherein the bond forms between Z and the nucleophile.

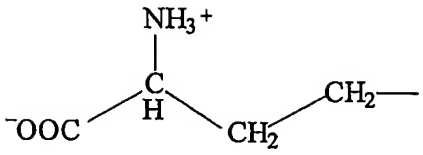
54. The method according to claim 53 wherein Z is boron.

55. The method according to claim 53 wherein Z is carbon.

56. A method of inhibiting γ-glutamyl transpeptidase, comprising contacting γ-glutamyl transpeptidase with an effective inhibitory amount of a compound having the formula:

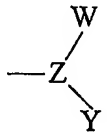
Q—T

wherein Q is



and T forms a non-labile bond with Q.

57. The method according to claim 56 wherein T is



wherein Z is C or B;

W is =O when Z is C, or –OH when Z is B; and

Y is –OH, lower alkyl, $-OPO_3$, $-(CH_2)_n-CO_2$, $-(CH_2)_n$-aryl, or $CR_5R_6R_7$; where

R_5 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

R_6 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl;

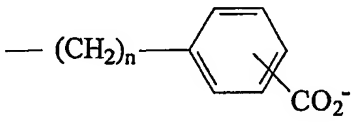
R_7 is H, halogen, $-PO_3H_2$, $-(CH_2)_n-CO_2$, or $-(CH_2)_n$-aryl; and

where at least one of R_5, R_6 or R_7 is halogen, and n is 1-8.

58. The method according to claim 57, wherein the aryl is benzyl.

59. The method according to claim 58, wherein the benzyl is a substituted benzyl.

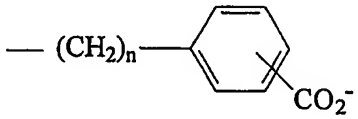
60. The method according to claim 59, wherein the substituted benzyl is



61. The method according to claim 57, wherein Z is B, W is –OH, and Y is –OH.

62. The method according to claim 57, wherein Z is B, W is –OH, and Y is $-(CH_2)_n-CO_2$, wherein n is 1.

63. The method according to claim 57, wherein Z is B, W is –OH, and Y is



wherein n is 1.

64. The method according to claim 57, wherein Z is C, W is =O, and Y is $CR_5R_6R_7$, wherein R_5, R_6, and R_7 are halogen.

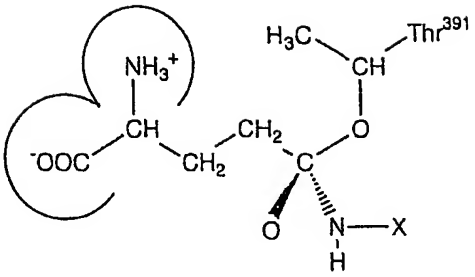
65. The method according to claim 57, wherein Z is C, W is =O, and Y is $CR_5R_6R_7$, wherein R_5 is $-OPO_3$ and R_6, and R_7 are halogen.

66. The method according to claim 57, wherein Z is C, W is =O, and Y is $CR_5R_6R_7$, wherein R_5 is $-(CH_2)_n-CO_2$, n is 1, and R_6 and R_7 are halogen.

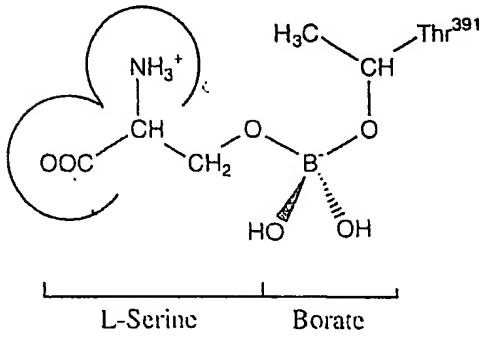
67. The method according to claim 56, wherein the compound is a reversible inhibitor of the γ-glutamyl transpeptidase.

68. Any of the compounds of the methods of claims 1-67.

A)


H3C
Thr391
CH
NH3+
CH
CH2
O
-OOC
CH2
C
O
N
X
H

B)


H3C
Thr391
CH
NH3+
CH
O
O
OOC
CH2
B
HO
OH
L-Serine
Borate

C)

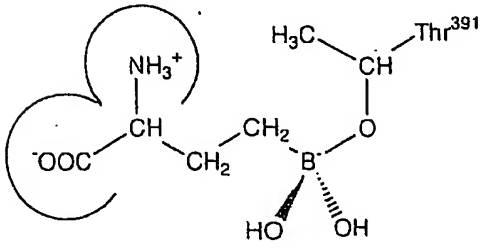

H3C
Thr391
CH
NH3+
CH
CH2
O
-OOC
CH2
B
HO
OH

FIG. 1

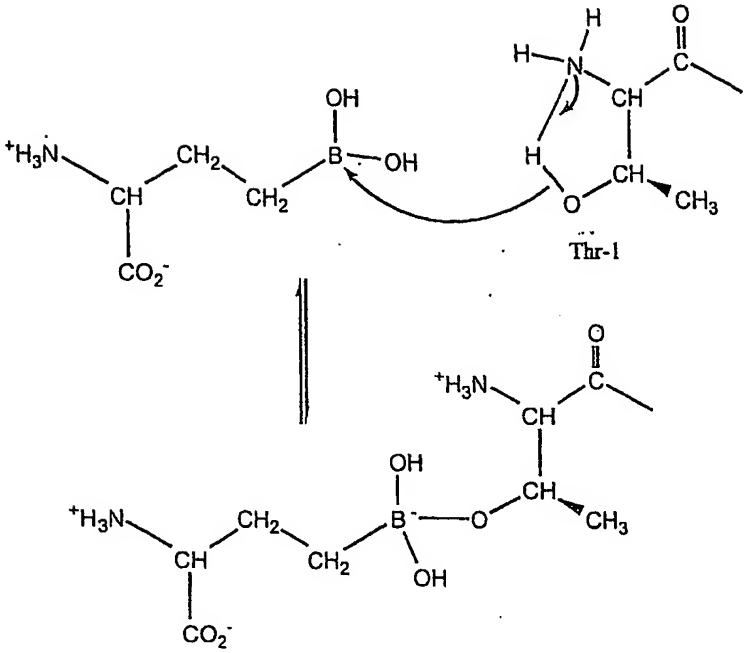


FIG. 2

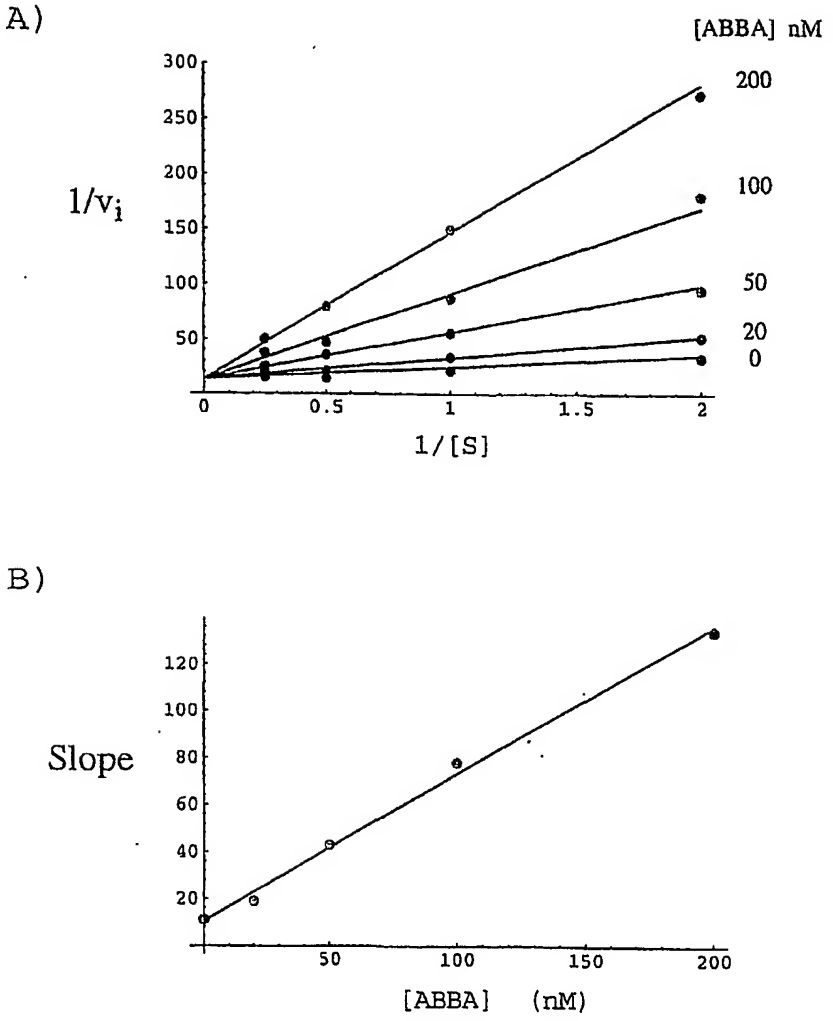

A)
[ABBA] nM
1/v_i
300
250
200
150
100
50
200
100
50
20
0
0
0.5
1
1.5
2
1/[S]
B)
Slope
120
100
80
60
40
20
50
100
150
200
[ABBA] (nM)

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/31225

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07F5/02 C07F9/38 C07C229/22 A61K31/14 A61K31/195
A61K33/22 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07F C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRIFFITH, OWEN W. ET AL: "Selective inhibition of.gamma.-glutamyl-cycle enzymes by substrate analogs" PROC. NATL. ACAD. SCI. U. S. A., vol. 74, no. 8, 1977, pages 3330-3334, XP001050456	68
A	the whole document	1,56
X	WO 99 09825 A (ELAN PHARM INC) 4 March 1999 (1999-03-04)	68
A	page 19 -page 20; table 4	1,56
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2002	25/01/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bader, K

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INTERNATIONAL SEARCH REPORT

In ›nal Application No
PCT/US 01/31225

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	STEIN, ROSS L. ET AL: "Slow-binding inhibition of.gamma.- glutamyl transpeptidase by.gamma.-boroGlu" BIOCHEMISTRY, vol. 40, no. 19, 2001, pages 5804-5811, XP001050567 the whole document	1-68
P,X	LONDON, ROBERT E. ET AL: "Development and Evaluation of a Boronate Inhibitor of.gamma.- Glutamyl Transpeptidase" ARCH. BIOCHEM. BIOPHYS. , vol. 385, no. 2, 2001, pages 250-258, XP001050426 the whole document	1-68

INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/US 01/31225

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9909825 A	04-03-1999	WO 9909825 A1	04-03-1999
		US 6310093 B1	30-10-2001